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Typed or Printed Name	Kimberly W. Zuehlke		
Signature		**Date**	

DECLARATION OF STEVEN L. GATTON UNDER 37 C.F.R. §1.132		
	Atty Docket	OLIG-0004
	First Named Inventor	Roderic M.K. Dale et al.
	Application Number	09/223,957
	Filing Date	December 31, 1998
	Group Art Unit	1623
	Examiner Name	G. Kunz
	Title	METHOD FOR NUCLEIC ACID PREPARATION

Dear Sir:

1. I, STEVEN L. GATTON, declare and say I am a resident of Lake Oswego, Oregon. My residence address is 18990 SW Pilkington Road, Lake Oswego, Oregon 97035-8130 U.S.A.

2. I hold a Bachelor degree in chemistry which I received from Muskingum College in May, 1976. I further hold an M.S. in chemistry which I received from Bowling Green State University in June, 1982. I am currently employed in the position of Director, Quality Assurance, and Safety/Chemical Hygiene Officer at the company Oligos Etc., Inc., 9775 SW Commerce Circle, Building C6 Wilsonville, Oregon 97070-0727. I am an expert in the fields of nucleic acid chemistry and oligonucleotide production and purification.

3. I am an inventor of the claims of the above-identified patent application. I directed others and personally performed the research leading to the invention disclosed and claimed therein.

4. I have read the Final Office Action dated August 17, 1999.

5. The following provides the evidence requested by the Examiner.

Summary of Hamilton PRP-1 vs Waters Prep C18

C18 columns and PRP-1 columns were compared for capacity limitations and efficacy in desalting and concentrating oligonucleotides following purification procedures. The columns used for this study, both C18 and PRP-1, were prepared using Millipore-Amicon Vantage 1.6 cm ID glass columns, poured to a height of 30 cm. Column volume of each column was calculated to be 60 mL.

The following results were obtained:

A. The Waters Prep C18 stationary phase cannot be used to load samples from an alkaline (pH 10-14) SAX purification without first being neutralized to less than pH 8. This introduces an additional step into the process which the PRP does not require. As such Examples 1 and 2 cannot be performed using the C18 phase without modifying the protocol by adding an additional neutralization step. In addition, it has been reported (Sofer et al., Process Chromatography: A Practical Guide, Academic Press, San Diego, California (1989), p. 93-105, copy provided for Examiner) that chromatography media that are compatible with sodium hydroxide at elevated pH are most suitable for production-scale work. This allows sanitization of the chromatography phase without the use of bacteriostatic additives. High pH (13-14) is also capable of removing endotoxins from chromatography media. Since this is within the PrP-1 working pH range and well outside that of the Prep C18, the PRP has a clear advantage over the Prep C18 for large-scale processes used for drug manufacture.

B. The pH range of the Waters Prep C18 phase as determined by the manufacturer not only does not permit the direct use of alkaline pH during loading, but it also precludes use of the C18 phase in acidification of acid-resistant oligonucleotides as demonstrated in Example 3. The pH of the acidifying solution is 1.5, which is below the lower limit of pH 3 of the C18. In addition, the typical loading range of oligonucleotide onto the PRP increases over 3-fold when performed at pH 1-2 rather than at pH 7. As written, Example 3 cannot be done on the Waters Prep C18 without sustaining damage to the phase. Example 3 is quite simple to perform using PRP phase.

C. The Waters Prep C18 phase leaches absorbed oligonucleotide during the wash that is intended to remove residual salt from the SAX purification. This makes the determination as to when the salt has been removed problematic. The PRP cleanly holds its absorbed material, and allows the column to be washed to very low levels of conductivity without loss of material. This feature makes the PRP much easier for the operator and/or automated equipment to assess the endpoint of the washing process and to start the elution.

D. The Waters Prep C18 is not compatible with the water-ethanol solvent system as practiced in all Examples of the application. The packing of the C18 columns used in this study cracked and channeled very badly during the elution. As a result, the columns required repacking before subsequent use. This problem has not been observed with the PRP, and columns have used for dozens of procedures without repacking.

E. Finally, the PRP column was able to retain a capacity of 4788 A_{260} for a 40-mer, compared to only 2880 A_{260} for a 40-mer with the C18 column. Thus the PRP column had a capacity 60% greater than the C18 column of approximately the same size and volume. This is a significant advantage in large scale production of oligonucleotides, as the fewer and/or smaller PRP columns would be required to desalt and concentrate synthesized oligonucleotides in large-scale preparations.

To summarize these results, the side-by-side comparison of the two columns are as follows:

stationary phase	Acid resistant	Base resistant	Operating pH Range	Capacity of 40-mer	Leaching during wash
PRP-1	Yes	Yes	0-14	4788 A_{260}	No
C18	No	No	3-8	2880 A_{260}	Yes

Thus, the PRP columns provide improved and unexpected results as exhibited by results including 1) improved pH range of use; 2) increased capacity; and 3) decreased leaching during the wash step as compared to the C18 columns.

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code, and that such will false statements may jeopardize the validity of the application or any patent issuing thereon.

1/6/2000
Date

Steven L. Gatton, ~~Ph.D~~ M.S.

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